Passive Smoking Affects Endothelium and Platelets

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• Blood was obtained before and after ten healthy male nonsmokers sat for 20 minutes in open hospital corridors beside men who were already there smoking by their own initiative. Mean values before and after passive smoking were 0.57 and 0.78 for the platelet aggregate ratio, 2.8 and 3.7 per counting chamber for the endothelial cell count, 0 and 2.8 ng/mL for the plasma nicotine concentration, and 0.9% and 1.3% for the carboxyhemoglobin level. No variable changed significantly during control periods in which the subjects sat in a room where smoking was prohibited. Passive exposure to tobacco smoke affected the endothelial cell count and platelet aggregate ratio in a manner similar to that previously observed with active smoking.

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Cigarette smoking has a strong epidemiologic association with atherosclerosis, myocardial infarction, occlusive peripheral vascular disease, and stroke.[1-4] Both endothelial damage and platelet activation are thought to be important in the pathogenesis of atherosclerosis and arterial thrombosis[5-8] and to occur as a response to cigarette smoking. Pterovsky and Hladovec[9] reported that the concentration of anuclear carcasses of endothelial cells in venous blood increased after cigarette smoking. Their observations have been confirmed in our laboratory, where the mean endothelial cell counts of healthy male and female naive smokers approximately doubled after smoking two tobacco cigarettes and did not change significantly after sham smoking.[8] We observed similar effects when male habitual smokers with[8] and without[10] coronary artery disease smoked. Enhanced platelet aggregate formation after active smoking has been observed in several studies.[8,10-12]

Passive smoking involves breathing both sidestream smoke that goes directly into the air from the burning end of tobacco products and mainstream smoke after it has been exhaled by smokers. Sidestream smoke has higher concentrations of several potentially noxious compounds than mainstream smoke, with the ratio of sidestream to mainstream carbon monoxide reported to be 4.7.[13] The present study was done to determine whether passive smoking in a naturally occurring environment has acute effects on the endothelium and platelets similar to those of active smoking.

METHODS

Subjects and Experimental Design

After an overnight fast, ten healthy male medical students and physicians, who were nonsmokers and had a mean age of 28 years (age range, 23 to 49 years), participated in two 20-minute experimental periods. The experiments took place in the morning before work to avoid exposure to environmental smoke before the experimental periods began. The periods were separated by one week, with five men having a control period first and five men having a passive smoking period first. The men did not take medicines on a regular basis and were prohibited from taking aspirin or other nonsteroidal anti-inflammatory agents from ten days before the first experimental period until completion of the second period. Control periods consisted of sitting in the laboratory where smoking was prohibited, and passive smoking periods consisted of sitting where several patients had come to smoke of their own accord in chairs placed against the length of one wall opposite elevators where a 1110 × 480-cm atrium with a 240-cm ceiling connected with corridors at each end by a 225-cm opening. When there was an unoccupied seat between two men who were smoking, the experimental subject sat there. Sometimes, the subject sat beside only one man who was smoking, because there was no unoccupied seat between two smokers. Antecubital venipunctures were done in the laboratory before and after each experimental period to obtain blood for determination of the platelet aggregate ratio, the endothelial cell count, the plasma nicotine concentration, and the carboxyhemoglobin level.

Platelet Aggregate Ratio

Our modification[8] of the method of Wu and Hoak[14] was used. The method is based on the ratio of the platelet count of platelet-rich plasma, prepared from blood that had been mixed immediately after venipuncture with a solution containing edetic acid (EDTA) and formaldehyde, to that of platelet-rich plasma prepared in the same manner, except for the absence of formaldehyde. Wu and Hoak[14] theorized that platelet aggregates circulating in blood are fixed when drawn into a solution that contains formaldehyde and edetic acid and break apart when drawn into a solution that contains edetic acid without formaldehyde. The data of others[15,16] suggest that the platelet aggregate ratio is also influenced by aggregates formed during the withdrawal of blood through a needle and tubing. In any case, a decrease in the platelet aggregate ratio reflects an increased formation of platelet aggregates. In the present study, the length of the plastic tubing through which blood

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was withdrawn was 8.9 cm rather than the 30-cm length used in our previous studies.[9]

Endothelial Cell Counts (Counts of Anuclear Endothelial Cell Carcasses)

The method of Hladovec and Rossmann[11] was used. Nine milliliters of venous blood was collected in a siliconized centrifuge tube that contained 1 mL of 3.8% trisodium citrate and mixed. Centrifugation at 4°C and 395 g (middle of the tube) for 20 minutes removed the erythrocytes and leukocytes. One milliliter of the supernatant was mixed with 0.2 mL of adenosine 5'-diphosphate, disodium salt (1 mg/mL) and mechanically shaken for ten minutes. Another centrifugation at 395 g for 20 minutes removed the platelet aggregates. The supernatant was then centrifuged at 2100 g for 20 minutes. After suspension of the sediment in 0.1 mL of physiologic saline by stirring with a siliconized glass rod, four Neubauer chambers were filled with the suspension, and the endothelial cells were counted using phase-contrast microscopy. Results were expressed as the mean cell count of the four 0.9-μL chambers.

On two occasions, we evaluated the possibility that the cells counted as endothelial cells might instead be megakaryocyte fragments. The cells were transferred from the saline suspensions to glass slides by centrifugation (Cytospin, Shandon Southern Instruments, Inc, Birmingham, Ala). The slides were incubated for 45 minutes with mouse monoclonal anti-human platelet glycoprotein Ib antibody (Dako Corp, Santa Barbara, Calif) that was diluted in phosphate-buffered saline to a concentration of 16.5 μg of antibody protein per milliliter, washed with phosphate-buffered saline, incubated for 45 minutes with goat anti-mouse IgG fluorescein conjugate (Boehringer Mannheim, Indianapolis) that was diluted to a concentration of 94 μg of antibody protein per milliliter, and washed again. Fluorescence microscopy revealed no fluorescence of the endothelial cell preparations, while simultaneously processed slides on which human bone marrow aspirates had been smeared showed strong fluorescence of large cells that were presumed to be megakaryocytes.

Plasma Nicotine

A portion of the platelet-rich plasma, prepared in the first centrifugation in the procedure for endothelial cell counting, was kept frozen at −80°C until it was thawed for extraction and preparation for gas chromatography by the method of Feyerabend and Russell,[12] using an instrument that was equipped with a nitrogen-phosphorus detector (Aerograph 1400, Varian Instruments Division, Walnut Creek, Calif). The length of the column was 90 cm. Column temperature was 150°C. The method was not otherwise modified from the original.[12] The means of duplicate assays of each sample of plasma were used for statistical analysis.

Carboxyhemoglobin

Blood was taken into a heparinized syringe for determination of the carboxyhemoglobin level by spectrophotometry (IL-282 CO-oximeter, Instrumentation Laboratories, Lexington, Mass).

Statistical Analysis

Two-tailed Wilcoxon signed-rank tests were used to determine the significance of the differences between the means of the paired variables shown in the Table and in Figs 1 through 4. Confidence intervals of the differences were calculated according to Gardner and Altman.[13] The Spearman rank correlation coefficient was used as a measure of the association between variables.

RESULTS

The Table shows the mean values of each variable before and after the control period. No significant differences occurred ($P>.2$ for each comparison).

Figure 1 shows that the platelet aggregate ratio of each of the ten subjects was lower after than before passive smoking. The mean values (±1 SD) were 0.87 ± 0.06 before and 0.78 ± 0.07 after passive smoking, with a mean difference of 0.09 and a 95% confidence interval of 0.03 to 0.15.

Figure 2 shows that the endothelial cell count was always higher after than before passive smoking. Mean values (±1 SD) before and after were 2.8 ± 0.9 and 3.7 ± 1.1 per counting chamber, respectively, with a mean difference of 0.9 per chamber and a 95% confidence interval of 0 to 1.8 per chamber.

Mean Values (± 1 SD) of Variables Before and After Control Period

Variable	Mean (± 1 SD) Before	After
Platelet aggregate ratio	0.88 (± 0.05)	0.88 (± 0.04)
Endothelial cell count	2.2 (± 0.8)	2.3 (± 1.0)
Plasma nicotine concentration, ng/mL	0	0
Blood carboxyhemoglobin level, %	1.1 (± 0.8)	1.2 (± 0.7)

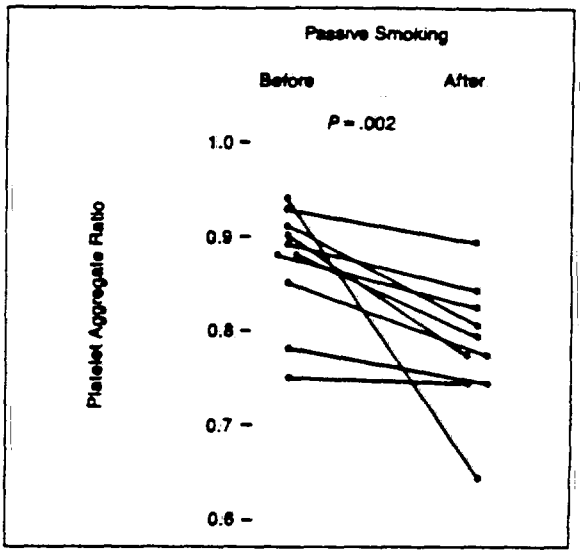

Fig 1.—Platelet aggregate ratios before and after passive smoking.

Figure 3 shows that nicotine was not detectable in the plasma of any subject before passive smoking and was present in the plasma of all but one subject after completion of the 20-minute period of passive smoking. The mean concentration (±1 SD) after passive smoking was 2.8 ± 1.2 ng/mL.

Figure 4 shows that the carboxyhemoglobin level was higher after passive smoking in all but one subject, whose value was unchanged. Mean values (±1 SD) were 0.9% ± 0.3% before and 1.8% ± 0.6% after passive smoking, with a mean difference of 0.4% and a 95% confidence interval of 0% to 0.8%.

After passive smoking, the percent carboxyhemoglobin level did not correlate significantly ($P>.60$) with the platelet aggregate ratio or the endothelial cell count. The correlation coefficient between the change in the carboxyhemoglobin level from before to after passive smoking and the corresponding change in the endothelial cell count was .78 ($P<.01$), while the change in the carboxyhemoglobin level was not significantly ($P>.30$) correlated with that of the platelet aggregate ratio. Neither the plasma nicotine concentration after passive smoking nor its change from

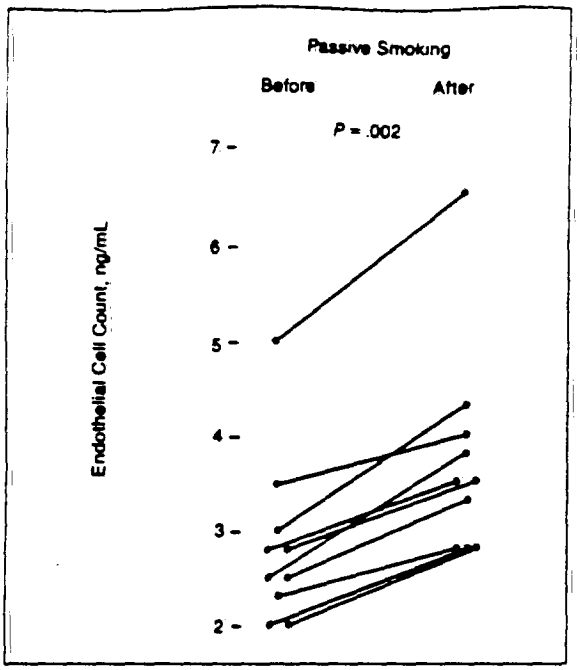

Fig 2.—Endothelial cell counts before and after passive smoking.

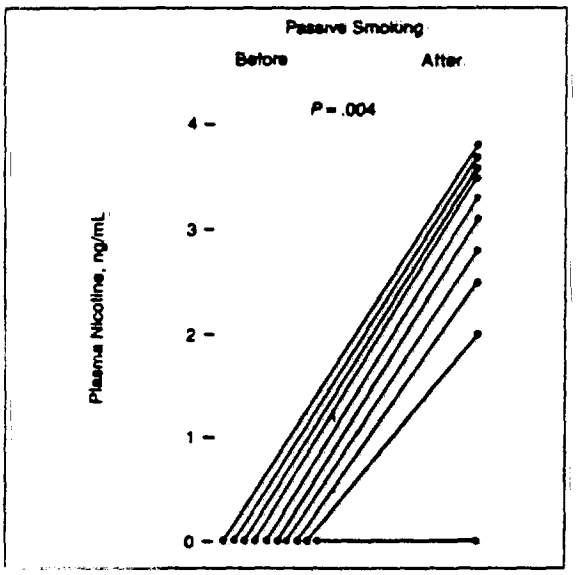

Fig 3.—Plasma nicotine concentrations before and after passive smoking.

before to after passive smoking was significantly ($P>.20$) correlated with the corresponding values of the platelet aggregate ratio or the endothelial cell count.

COMMENT

Hladovec and Rossmann[17] described a simple method for the quantitation of anuclear carcasses of endothelial cells in blood. The identity of endothelial cells was based on their morphologic similarity to cells that were scraped from the endothelium, with the exception that the cells isolated from blood had no nuclei. Takahashi and Harker[20] added cultured human endothelial cells to blood and recovered them from mononuclear cell fractions in which they were identified by the presence of factor VIII–related antigen as a cell marker. They detected no endothelial cells in mononuclear cell fractions of venous blood from ten normal subjects. Using the method of Hladovec and Rossmann,[17] we[9,10] found endothelial cells in the venous blood of normal men and women and men with coronary artery disease. Since, like Hladovec and Rossmann,[17] we saw no nuclei in these cells, there is no conflict with the report of Takahashi and Harker[20] of the absence of endothelial cells from mononuclear cell fractions. In a previous study,[9] we identified the anuclear cells as endothelial by their fluorescence after incubation with fluorescein-labeled anti–human factor VIII–related antigen antibody and by the lack of fluorescence of the epithelium in simultaneously incubated sections of human skin.

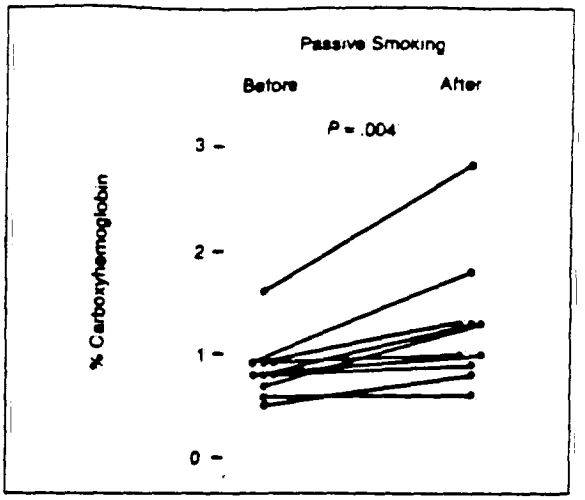

Fig 4.—Carboxyhemoglobin levels before and after passive smoking.

The present study, showing that brief passive exposure to tobacco smoke under naturally occurring environmental conditions has consistent acute effects on the endothelium and platelets, was limited to a group of ten healthy male nonsmokers. It seems likely that this small group may be representative of the general population since we observed similar effects of active smoking on healthy male and female naive smokers,[9] healthy male[10] and female (J.W.D. and L.S., unpublished data, 1982) habitual smokers, and male habitual smokers with coronary artery disease.[9,10] Other workers have shown that passive smoking by nonsmokers lowers platelet sensitivity to the antiaggregatory effect of prostacyclin.[21]

Although a statistically significant correlation between the change in the endothelial cell count and the change in the carboxyhemoglobin level from before to after passive smoking was found, there was not a significant correlation between these variables after passive smoking. We previously found that smoking tobacco cigarettes had a much greater effect on both platelets and the endothelium than did smoking cigarettes that contained no nicotine.[9] The relative importance of carbon monoxide, nicotine, and the many other components of tobacco smoke as causes of the

observed effects on platelets and the endothelium remains unclear.

The significance of enhanced platelet aggregate formation and an increased concentration of anuclear carcasses of endothelial cells in blood after passive smoking is not known. However, both platelet activation[4] and endothelial damage[5] are prominent among the mechanisms thought to be involved in atherosclerosis and arterial thrombosis. Epidemiologic studies are needed to determine whether repeated episodes of passive exposure to tobacco smoke during a period of years enhance the development of atherosclerosis and its complications in nonsmokers. A large Japanese study[22] indicated that nonsmoking wives of heavy smokers had a higher risk of developing lung cancer, while the husbands' smoking habit did not affect their wives' risk of dying of ischemic heart disease. Stock[23] suggested that Japanese people may be protected from fatal coronary heart disease by a high dietary intake of eicosapentaenoic acid. We hope that our work will be an impetus to the development of epidemiologic investigations of a possible relationship between passive smoking and vascular diseases in Western countries.

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